Going Retro: Ancient Viral Origins of Cognition

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In this issue of *Neuron*, Zhang et al. (2015) provide the first crystal structure of a domain Arc. These results confirm prior computational approaches that suggested Arc, a master regulator of vertebrate synaptic plasticity, was "domesticated" from Ty3/Gypsy retrotransposons.

The activity-regulated cytoskeletal protein Arc (also known as Arg3.1) is encoded by an immediate-early gene (IEG) discovered in two independent screens for neural novel genes induced by seizure. Initial excitement surrounding Arc stemmed from the discovery that its mRNA accumulates in recently active dendritic subregions, making it a potential molecular mediator of localized synaptic plasticity (Link et al., 1995; Lyford et al., 1995). Since its discovery, Arc has become appreciated as a master regulator of plasticity, critical for maintenance of long-term potentiation and both mGluR and NMDAR-dependent forms of long-term depression (Guzowski et al., 2000; Park et al., 2008). Additionally, Arc regulates homeostatic scaling of synaptic strength and is necessary for consolidation of memory in rodents (Guzowski et al., 2000; Shepherd et al., 2006).

In the past 20 years, knowledge of the cellular and molecular biology of Arc has grown steadily. However, since *Arc* is a single copy gene with minimal homology to other metazoan genes, understanding of the structural foundations of Arc-mediated processes has been extremely limited (Lyford et al., 1995). The earliest progress in understanding Arc structure came, surprisingly, from a computational analysis of the human genome that was searching for genes with homology to retroviral Gag proteins. Arc was one of over 100 human proteins predicted to have been "domesticated" from the retrotransposon remains of ancient viruses (Campillos et al., 2006). All of human experience, from life's most mundane tasks to the highest achievements in art and science, are rooted in our ability to reliably encode and store new information. The possibility that Arc, an absolutely indispensable component of learning and memory, has retroviral origins was surprising and intriguing. New support for this hypothesis comes in this issue, where Zhang et al. (2015) report the first crystal structure of an Arc domain.

Their study presents the crystal structure Arc amino acids (aa) 207–278 in complex with TARPγ2 and CamKII peptides and Arc aa 278–370 apoprotein. Full-length Arc did not crystalize. The two protein structures resemble the highly similar N-terminal domain (NTD) and C-terminal domain (CTD) of the bilobar retroviral Gag capsid domain (CA). As such, the authors refer to Arc 207–278 as the N-lobe and Arc 278–370 as the C-lobe. The two Arc lobes have high structural similarity to both HIV and Rous sarcoma virus (RSV) Gag CA, despite relatively low sequence homology, and are structurally divergent from other known proteins.

Examining features of Arc orthologs across various species paints a picture that Arc domestication took divergent paths during evolution. The authors show that Arc N-lobe contains a hydrophobic binding pocket that mediates its interaction with numerous synaptic proteins, including TARPγ2, an auxiliary AMPAR subunit. N-lobe substrate binding is a feature present only in higher vertebrates and confers on Arc a unique ability to regulate synaptic strength via interactions with plasticity-related substrates. Conversely, insect and fish Arc variants retain nucleotide binding and reverse transcriptase domains from retroviral Gag and Pol that are absent in the mammalian ortholog.

Among Arc's cellular functions is the endocytosis of certain proteins, notably AMPARs (Chowdhury et al., 2006). A long-standing question surrounding Arc is how does it provide target selectivity? The authors show that mutations that eliminate Arc N-lobe binding disrupt its interaction with TARPγ2 and prevent Arc overexpression from reducing surface TARPγ2 and GluA1 levels. Analysis of Arc substrate binding led the authors to identify a consensus N-lobe binding sequence that allows a priori predictions of novel Arc substrates. Phosphorylation of serine or tyrosine residues in this sequence in TARPγ2 inhibits Arc binding. The authors also demonstrate that Arc N-lobe binding is druggable. Arc and its signaling networks have been implicated in the etiology of multiple neurological disorders associated with cognitive disabilities (Korb and Finkbeiner, 2011). Compounds that selectively target Arc substrate binding could have therapeutic potential. For example, Angelman syndrome is thought to feature excessive Arc-mediated endocytosis of AMPARs (Greer et al., 2010). Inhibiting Arc N-lobe binding may permit the synapses of patients to function more normally.

In their discussion, the authors highlight a number of peculiar aspects of Arc biology that may be related to its retrotransposon origins. As in the main text, emphasis was placed on the viral genesis of Arc's synaptic functions, including

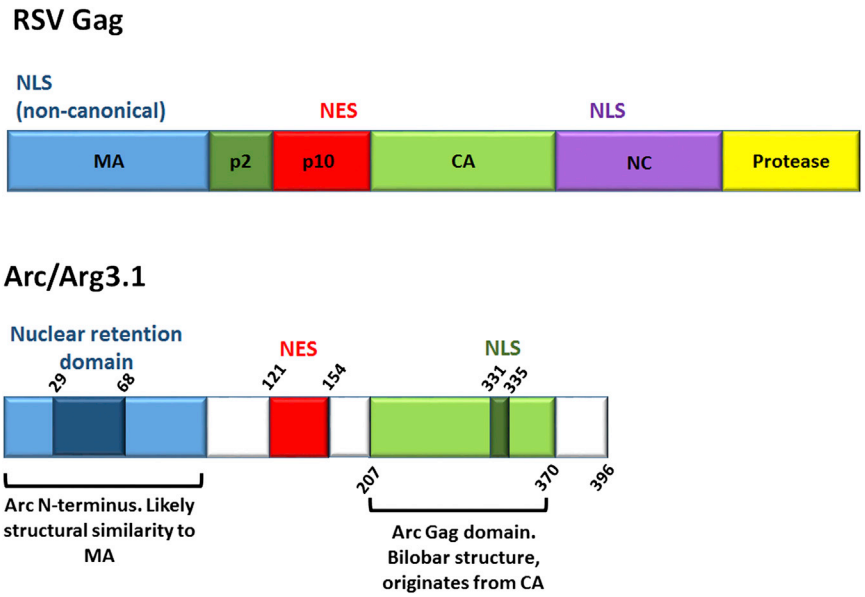

Figure 1. RSV Gag and Arc Share Similar Nuclear Localization Signals

RSV Gag has a non-canonical NLS in MA. Arc has a non-canonical NLS (termed a nuclear retention domain) near its N terminus, a region expected to share structural similarities to retroviral MA. Both RSV Gag and Arc have a canonical NLS near their C-terminal ends. The localization of these signals is not identical, as Arc does not have an NC. RSV Gag also has an NES in its p10 domain. Arc similarly has an NES between its putative MA- and CA-like domains. It is unknown whether the NES-containing region of Arc shares structural features of RSV p10. RSV Gag structure was adapted and modified from Parent (2011) under a Creative Commons license.

mechanisms of mRNA transport and local translation. Arc is also an important plasticity protein in the nucleus. Synaptic activity regulates its translocation between the cytoplasm and nucleus. Inside the nucleus, Arc promotes the formation of promyelocytic nuclear bodies, where it sequesters CBP and, in turn, suppresses transcription of GluA1 during homeostatic downscaling (Korb et al., 2013). Focusing on Arc as a nuclear protein with retrotransposon origins reveals some novel insights not presented by Zhang and colleagues. During replication, retroviral Gag mRNA is transcribed, shuttled to the cytoplasm, and translated into Gag polyprotein. The simple model that Gag polyproteins bind cytoplasmic retroviral RNA to begin assembly of a functional viral particle is false. Instead, in the case of RSV, HIV, and other retroviruses, Gag is initially trafficked back to the nucleus (Parent, 2011). Formation of a ribonucleoprotein complex with genomic retroviral RNA (gRNA) is thought to induce Gag dimerization, which exposes a nuclear export signal (NES). This drives nascent viral particles back to the cytoplasm for subsequent budding from the plasma membrane. Comparing the nuclear trafficking signals in RSV Gag to those identified in Arc reveals some striking similarities (Figure 1).

We previously identified three novel signals in Arc protein that control its nuclear-cytoplasmic localization. The first was a non-canonical nuclear localization signal (NLS), termed a "nuclear retention domain" (Korb et al., 2013). This signal occurs in the region of Arc expected to have evolved from the matrix domain (MA) of retroviral Gag. Remarkably, RSV MA also contains an atypical NLS. Arc also has a second, classical NLS in its C terminus (aa 331–335). Similarly, RSV Gag also has a classical NLS, though it is located just beyond the CA in the nucleocapsid domain (NC). In retroviruses NC binds RNA, promoting Gag dimerization and export. Although Arc lacks a NC, it is still capable of self-oligomerization (Myrum et al., 2015; Parent, 2011). What factors regulate this process, and whether it controls nuclear export of Arc are unknown. Finally, Arc contains a NES located near the center of the protein. This region corresponds spatially to the p2/p10 region of RSV Gag that also contains an NES (Butterfield-Gerson et al., 2006).

This study provides exciting new avenues for Arc research. First, crystal structures of two domains of Arc's C-terminal half offer strong evidence for its retroviral ancestry and reveal the structural basis of target binding. Appreciating its retrotransposon origins provides a new frame of reference for evaluating Arc regulation and function. The field should continue to attempt crystallization of full-length Arc or portions of the Arc N terminus (aa 1–206). Arc N-lobe is not the only portion of the molecule that binds proteins. Arc amino acids 89–199 interact with endophilin and are necessary for Arc's AMPAR endocytic function. This suggests that Arc might act as a scaffold, promoting endocytosis by bringing together the vesicle internalization apparatus and target proteins (e.g., TARPγ2-GluA1). Cooperative binding of targets by either side of Arc could explain how Arc mediates a variety of distinct molecular processes. In this model, the functional impact of Arc N-lobe binding to its various targets would depend largely on what is bound by the N-terminal half of the protein. From the predicted structure and location relative to retrotransposon CA, a portion of Arc N terminus (aa 1–206) is hypothesized to have evolved from retroviral MA (Campillos et al., 2006). Like Arc, retroviral MAs have coiled-coil domains and mediate protein lipid interactions (Matthews et al., 1994; Parent, 2011). However, unlike MA, Arc's interactions with lipid regulate endocytic processes rather than viral budding. A structural explanation for the difference will provide great insights into known functions of Arc. The authors also discovered an Arc N-lobe binding sequence and provided a list of potential synaptic targets. One of these, WAVE1, is an actin nucleator and may be critical to Arc's cytoskeletal functions. Additional characterization of these potential binding partners will expand our understanding of Arc biology at the synapse and the mechanistic basis of synaptic plasticity. Since Arc is also a nuclear protein, a similar search and validation of nuclear targets should be pursued. Finally, Arc N-lobe binding was shown to be inhibited by phenothiazine antipsychotics. Since Arc and its signaling pathways have been implicated in multiple

neurological disorders featuring cognitive deficits, development of more selective Arc inhibitors has exciting therapeutic potential. Given the large and growing list of Arc binding partners, the effects of inhibiting Arc could be variable and difficult to predict. Assessing efficacy in Angelman syndrome models would be, perhaps, the most reasonable starting point, as increased levels of Arc are directly implicated in its etiology.

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Balancing Excitation and Inhibition

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In this issue of *Neuron*, D'amour and Froemke (2015) examine how inhibitory spike-time-dependent plasticity (STDP) interacts with co-activated excitatory STDP to regulate excitatory-inhibitory balance in auditory cortex.

Cortical processing depends on glutamatergic excitatory synapses to propagate neural firing and on GABAergic inhibitory synapses to shape the temporal and spatial patterns of firing. In an active cortex, changes in excitatory synaptic drive are often matched by corresponding changes in inhibitory synaptic drive, supporting the notion that cortical processing depends critically on the balanced interplay of excitation and inhibition (E/I balance) (Isaacson and Scanziani, 2011), a balance that is dynamically maintained (Tao et al., 2014; Xue et al., 2014; Zhou et al., 2014). Indeed, alterations in the E/I balance impair essential features of the cellular response in sensory cortices, including dynamic range, stimulus selectivity, and gain control (Isaacson and Scanziani, 2011), and also impair learned performance in prefrontal cortex (Yizhar et al., 2011). E/I alterations have also been implicated in autism and schizophrenia. On the other hand, cortical circuits not only process information, but also store it as changes in the strength of glutamatergic connectivity, and this plasticity allows adaptive responses to altered sensory experience. Notably, in the cases examined, in the long run experience-dependent remodeling of the excitatory connectivity is accompanied by changes in inhibitory circuits such that the E/I is maintained (Froemke et al., 2007; House et al., 2011). Thus, adaptive cortical plasticity, for example, lowering the threshold for a particular sensory stimulus, might not compromise the conditions for processing other stimuli. At a synaptic level, these observations also raise the important question of whether mechanisms that allow plasticity of excitatory and inhibitory synapses can be coordinated. The answer is yes, as documented by the D'amour and Froemke analysis of spike-timing-dependent plasticity (STDP) in the auditory cortex reported in this issue of *Neuron* (D'amour and Froemke, 2015).

STDP is an attractive model of synaptic plasticity as it is induced by near-coincidental (within tens of milliseconds) pre- and postsynaptic activation. In most glutamatergic cortical synapses STDP tends to follow the Hebbian rule resulting in long-term potentiation (LTP) or depression (LTD) depending on whether the